

Assignment of Carbon-13 NMR Spectra via Double-Quantum Coherence

The spin-spin coupling between carbon-13 nuclei is a particularly useful property on which to base a determination of the network of linkages within an organic molecule. One-bond carbon-carbon couplings are readily distinguished from long-range couplings on the basis of their magnitudes, identifying adjacent carbon atoms in an unambiguous manner. For materials with the natural isotopic abundance (1%), it is sufficient to consider only pairs of interacting carbon-13 nuclei in any given molecule, so the corresponding spin systems are very simple: AX, AB, or A_2 .

Recent experiments (1-3) have exploited this principle through a technique which suppresses the strong resonances from isolated carbon-13 spins, revealing the weak satellite spectrum from molecules with two coupled carbon-13 spins. Good discrimination is achieved by excitation of double-quantum coherence (4-8), which exhibits a characteristic dependence on the phase of a radiofrequency pulse, different from that of single-quantum coherence or longitudinal magnetization. A phase-cycling procedure suppresses the unwanted strong signals leaving only signals derived from double-quantum coherence. Only the coupled carbon spins can generate such double-quantum signals.

Each carbon site may be directly coupled to as many as four other sites, and since the coupling constants are often very similar in magnitude, assignment to specific pairs of carbon atoms cannot always be made on the basis of coupling constants alone. Fortunately the coupled spins can be identified by means of a different criterion—the frequency of the double-quantum coherence, which is equal to the sum of the chemical shifts of the two carbon sites, measured with respect to the transmitter frequency. Since double-quantum coherence may not be observed directly (5) it is converted into transverse nuclear magnetization and its frequency is determined by means of a two-dimensional Fourier transform experiment (5, 9). This method has recently been used to establish the connectivity of the carbon atoms in 5 α -androstandane (3).

In its simple form the experiment uses the pulse sequence shown in Fig. 1a. The first three pulses serve to excite double-quantum coherence, which is allowed to evolve for a variable period t_1 , when the fourth pulse converts it back into detectable transverse nuclear magnetization. In this reconversion process, only the imaginary component of double-quantum coherence is recovered, leaving an ambiguity about the sense of precession of the double-quantum coherence during t_1 . In the resulting two-dimensional spectrum $S(F_1, F_2)$, the signs of the double-quantum frequencies are not determined, and this could be a critical problem for spectra of any complexity. The purpose of the present communication is to describe an extension of the method which allows the sense of this precession to be determined by detecting the

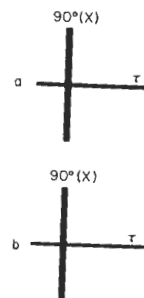


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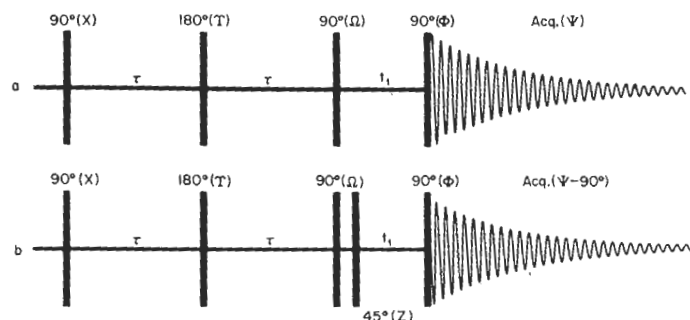


FIG. 1. Two stages of the sequence used to detect carbon-13 satellites in carbon-13 spectra while relating them to the characteristic double-quantum frequencies. The phase angles Υ , Ω , Φ , and Ψ are cycled as shown in Tables 1 and 2. The basic pulse sequence (a) is modified in (b) by the insertion of a $45^\circ(Z)$ pulse, together with a 90° clockwise shift of the receiver reference phase, in order to determine the sign of the double-quantum frequency.

real and imaginary components of the double-quantum coherence in quadrature. This is accomplished by making two measurements at each setting of t_1 , one with the simple sequence of Fig. 1a, the second with a modified sequence which examines the component in quadrature. The crucial step is to induce a 90° phase shift of the double-quantum coherence. In principle, this could be achieved by altering the phase of the first three pulses of the sequence, but since double-quantum coherence is twice as sensitive to radiofrequency phase shifts as single-quantum coherence, this would entail a 45° phase shift and consequent hardware modification (11).

An alternative solution is proposed here. It employs a composite sequence of three pulses which has the overall effect of rotating single-quantum transverse magnetization through 45° about the Z axis, while double-quantum coherence experiences a 90° rotation about Z (12, 13). This will be represented as a $45^\circ(Z)$ pulse. Provided that resonance offset effects and radiofrequency inhomogeneity effects can be neglected, it can be shown that

$$90^\circ(+X)\theta(+Y)90^\circ(-X) \equiv \theta(+Z), \quad [1]$$

$$90^\circ(-X)\theta(-Y)90^\circ(+X) \equiv \theta(+Z) \quad [2]$$

TABLE 1

PHASE CYCLING OF THE "RECONVERSION PULSE" $90^\circ(\Phi)$ AND THE RECEIVER REFERENCE PHASE (Ψ)

Φ	Ψ
$+X$	$+X$
$+Y$	$-Y$
$-X$	$-X$
$-Y$	$+Y$

TABLE 2
PHASE CYCLING OF THE REFOCUSING PULSE,
180°(Y), THE CONVERSION PULSE, 90°(Ω),
AND THE SENSE OF THE ACQUIRED
SIGNAL (Acq.)

Y	Ω	Acq.
±X	+X	+
±X	-X	-
±Y	+X	-
±Y	-X	+

for any value of θ . These identities are readily appreciated by considering separately the effect of the three rotations on M_z , M_x , and M_y . The quantity M_z remains unchanged while M_x becomes $(M_x \cos \theta + M_y \sin \theta)$, and M_y becomes $(-M_x \sin \theta + M_y \cos \theta)$.

Figure 1a shows the basic pulse sequence (1, 2). Optimum transfer into double-quantum coherence requires $\tau = 1/(4J_{CC})$ so that a choice of $\tau = 6$ msec ensures good conversion for direct C-C couplings in saturated systems, and only weak conversion for the small long-range couplings. Quadrature detection in the F_1 dimension can be achieved by inserting a 45°(+Z) pulse at any point during the evolution period t_1 (Fig. 1b) together with a 90° (clockwise) shift of the receiver reference phase. It is convenient to insert the composite three-pulse sequence immediately after the 90°(±X) pulse which first creates double-quantum coherence. This allows four pulses to be consolidated into two:

$$90^\circ(+X)90^\circ(-X)45^\circ(-Y)90^\circ(+X) \equiv 45^\circ(-Y)90^\circ(+X), \quad [3]$$

$$90^\circ(-X)90^\circ(+X)45^\circ(+Y)90^\circ(-X) \equiv 45^\circ(+Y)90^\circ(-X). \quad [4]$$

The choice is determined by the sense of the first pulse.

In sequences (a) and (b) of Fig. 1, phase cycling is used to cancel unwanted signal components, so that before the two quadrature components are combined they contain negligible contributions from molecules with only isolated carbon-13 spins. It can be shown (1) that the desired signal (derived from double-quantum coherence) cycles in the opposite sense to the unwanted "single-quantum" signal as the phase Φ of the last 90° pulse is incremented in 90° steps. Consequently the receiver reference phase Ψ is cycled according to Table 1. This four-step cycle is essential to the process of discrimination between coupled and isolated spins. A higher degree of suppression can be achieved by further

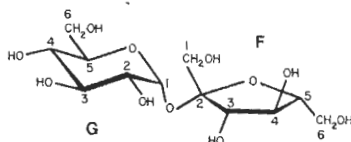
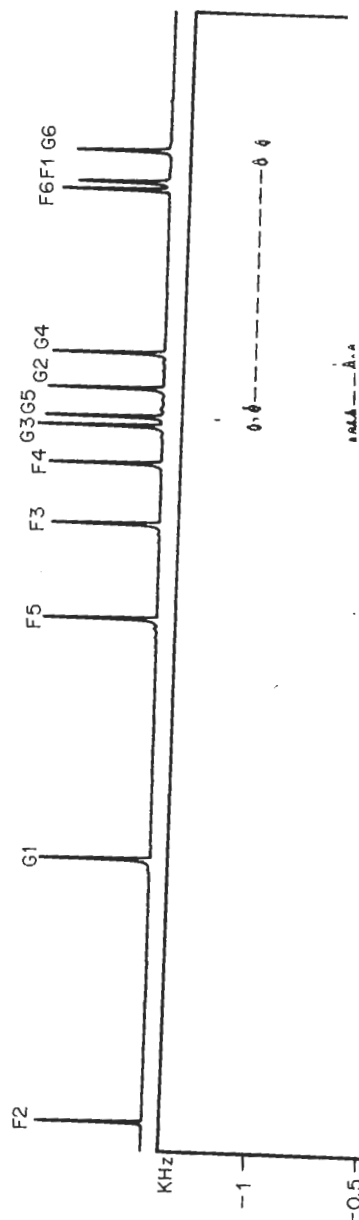


FIG. 2. The numbering scheme used for the carbon atoms in sucrose.



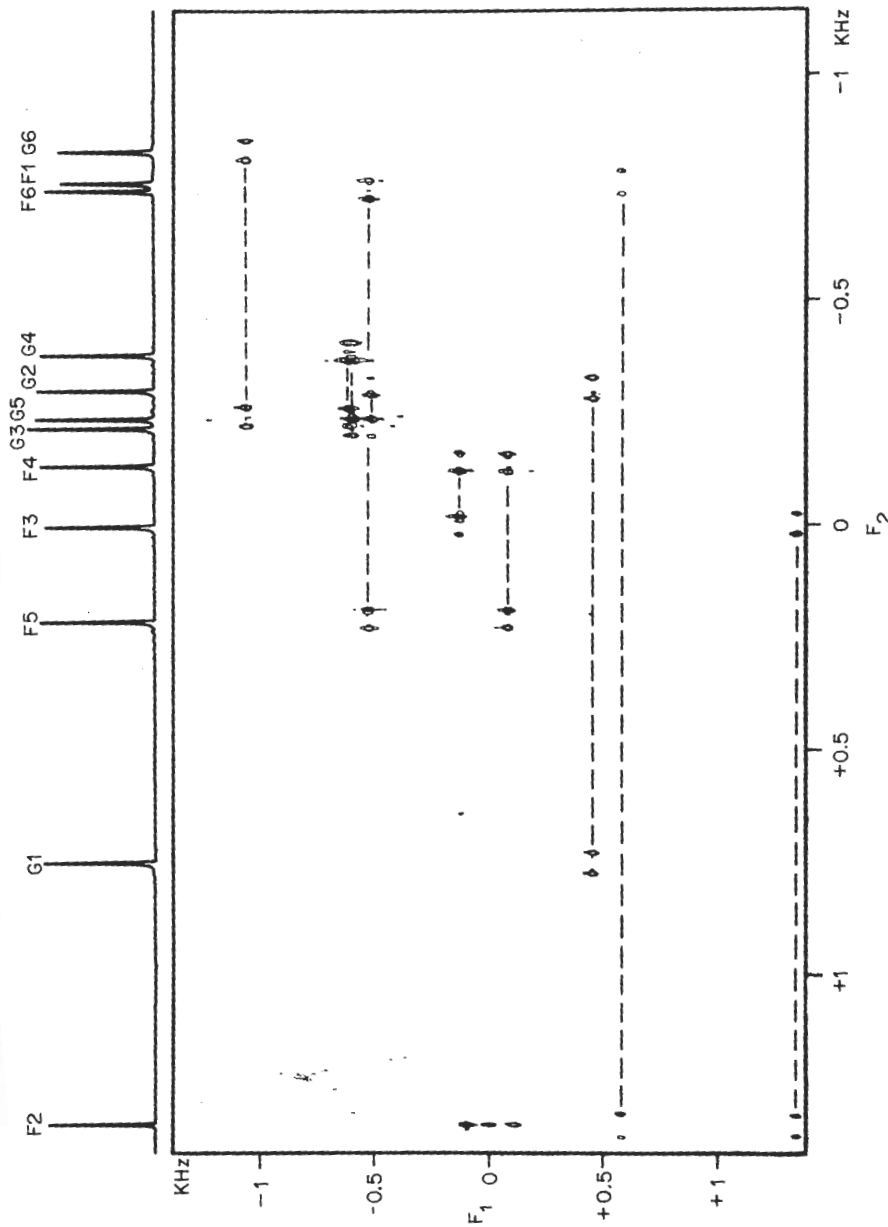


FIG. 3. The two-dimensional Fourier transform carbon-13 spectrum of sucrose obtained as described in the text. The AX- or AB-type satellite spectra are the four-line patterns joined by broken lines; their centers of gravity lie on a line with $\Delta F_1/\Delta F_2 = 2$. The conventional carbon-13 spectrum running along the top of the diagram has been assigned by noting which resonances have a direct carbon-carbon spin coupling. The double-quantum frequencies appear in the F_1 dimension.

phase cycling (Table 2) which, including the four-stage "CYCLOPS" sequence (14), gives a total of 256 distinct stages in the complete cycle.

As an illustration of the method the carbon-13 satellites of the carbon-13 spectrum of sucrose (Fig. 2) were investigated. The spectrometer was a Varian XL-200 operating at 50 MHz with a 16-mm sample at 70°C, and the total experimental time was 11 hr. The two-dimensional spectrum $S(F_1, F_2)$ is displayed in the form of an intensity contour plot (15). The F_2 dimension corresponds to the conventional carbon-13 spectrum except that the strong lines from isolated nuclei are suppressed, leaving only the weak satellite signals. Since the proton resonances are broadband decoupled throughout, these are four-line spectra of the AX or AB type. Ten such spectra are visible in Fig. 3, marked by the broken lines; four of them show the characteristic AB intensity pattern. The F_1 dimension separates these spectra according to their individual double-quantum frequencies, thus identifying them unequivocally. Since the double-quantum frequency is equal to the sum of the appropriate carbon-13 shifts, the centers of gravity of all AX or AB spectra lie on a diagonal with $\Delta F_1/\Delta F_2 = 2$. This additional constraint could be useful for identifying incompletely suppressed "single-quantum" signals.

The resonance of the quaternary carbon of sucrose (F2) was identified by its long spin-lattice relaxation time. Inspection of Fig. 3 reveals that this resonance is directly coupled to two others, which may then be labeled F1 and F3. Resonance F1 shows no other direct coupling, but F3 is clearly coupled through a single bond to another resonance, now labeled F4, and hence to F5 and F6. None of the glucose ring (G) resonances are involved in this coupling scheme. However, the three CH_2 sites are readily identified and since F1 and F6 have been assigned to the fructose ring, G6 can be identified. The chain of linkages G6-G5-G4-G3-G2-G1 may then be deduced from Fig. 3 by inspection: the fact that three AB quartets fall close together presents no particular difficulty. The assignment agrees with that of Pfeffer *et al.* (16).

The double-quantum technique provides a simple but powerful procedure for determining the connectivity of the carbon atoms in an organic molecule. (In principle C-N-C or C-O-C linkages could be "bridged" by searching out the long-range C-C couplings by an extension of the method, using longer delay periods τ .) For materials with natural isotopic abundance the sensitivity is necessarily low, but the technique has the advantage of great simplicity because it examines isolated pairs of carbon-13 spins, and thus builds up a picture of the carbon skeleton in very simple steps.

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